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## Prospective relation of C-reactive protein with type 2 diabetes

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## OBSERVATIONS

## Analysis of the Agreement Between the World Health Organization Criteria and the National Cholesterol Education Program-III Definition of the Metabolic Syndrome

Results from a population-based survey

**O**ur objective was to evaluate the diagnostic proficiency of the World Health Organization (WHO) and the National Cholesterol Education Program (NCEP)-III definitions (1,2) for the metabolic syndrome in a Mexican nationwide, population-based survey. Details of the sampling procedures have been previously described (3). The population was composed of 2,158 men and women aged 20–69 years sampled after a 9- to 12-h fasting period. For the WHO criteria, insulin resistance was diagnosed if a nondiabetic case had fasting insulin concentrations  $\geq 126$  pmol/l (21  $\mu$ U/ml) ( $>75$ th percentile in Mexican adults). The age-adjusted prevalence was 13.61% for the WHO criteria ( $n = 268$ ) and 26.6% for the NCEP-III definition ( $n = 574$ ). After excluding patients with diabetes, the prevalence was 9.2 and 21.4%, respectively. The agreement between the definitions was assessed in 1,969 subjects; 189 cases were eliminated due to the lack of a urine sample.

The number of abnormal cases was lower using the WHO criteria. Only 237 of the 545 subjects (43.4%) who fulfilled the NCEP criteria were diagnosed as affected using the WHO definition. Just 16 of 253 cases (6.3%) detected by the WHO definition did not fulfill the NCEP definition. The agreement between the criteria was moderate ( $\kappa = 0.507$ ). On the other hand, the subjects diagnosed using the WHO recommendations had a worse profile than the cases detected by the NCEP-III definition only—they had a

higher BMI and higher non-HDL cholesterol, triglyceride, and glucose concentrations. The demonstration of insulin resistance among the nondiabetic population caused the lack of agreement in 202 of the 242 cases that fulfilled the NCEP definition but failed the WHO criteria. Other reasons for disparity were the higher thresholds used by the WHO criteria; these differences explained the lack of agreement in 66 of the 152 cases with diabetes.

In conclusion, the prevalence of the metabolic syndrome is influenced by the selection of the diagnostic criteria. The WHO criteria identified a lower number of cases than the NCEP-III definition. These differences were explained mainly by the inclusion of abnormally high insulin concentrations as a diagnostic criterion. However, the presence of insulin resistance may help to identify patients more severely affected (4).

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## Complete Blockade of the Renin-Angiotensin System in Patients With Advanced Diabetic Nephropathy

**W**e read with interest the article by Rossing et al. (1) in the January 2002 issue of *Diabetes Care*. We had previously assessed the effect of complete blockade of the renin-angiotensin system (RAS) in patients with advanced diabetic nephropathy (type 2 diabetes) and severe proteinuria (2). To do so, we studied 10 patients on prior treatment with ACE inhibitors at recommended doses, to which 50 mg/day of Losartan was added.

After 3 months of the combined therapy, urinary protein excretion decreased from a mean of 6.9 (95% CI 4.3–9.6) to 5.8 g/24 h (3–8.6) ( $P = 0.025$ ) and, with the exception of two patients, was reduced in all cases. The proteinuria/urinary creatinine ratio also decreased from 7.6 (4.2–11.05) to 6.0 g/g (3.2–8.7) ( $P = 0.02$ ). In one patient in which proteinuria did not vary, the proteinuria/creatinine ratio also decreased, reflecting a possible error in the quantification of the patient's proteinuria instead of a lack of response to the treatment. The glomerular filtration rate (GFR), calculated using the mean of creatinine and urea clearance, a measurement that avoids the overestimation that creatinine clearance produces in the GFR (3), decreased slightly from  $21.5 \pm 8.5$  to  $19.9 \pm 7.8$  ml/min ( $P = 0.158$ ). An interesting decrease in total cholesterol from  $6.21 \pm 1.03$  to  $5.33 \pm 1.27$  mmol/l ( $P = 0.036$ ) was observed. Total proteins, serum albumin, protein intake, and mean arterial pressure (MAP) were not significantly modified. Only one patient had hyperkalemia (from 5.1 to 7.2 mmol/l). No secondary effects were seen in the other patients. At 12 months of treatment, proteinuria decreased to 3.4

g/24 h and the proteinuria/urinary creatinine ratio to 3.6. GFR decreased by  $0.83 \text{ ml} \cdot \text{min}^{-1} \cdot \text{month}^{-1}$  in the first 6 months and only fell by  $0.18 \text{ ml} \cdot \text{min}^{-1} \cdot \text{month}^{-1}$  during the last 6 months. Two patients received the combined treatment for >42 months. During the first 18 months, proteinuria decreased by 74 and 67% in each case; GFR fell by 0.5 and  $1 \text{ ml} \cdot \text{min}^{-1} \cdot \text{month}^{-1}$ , respectively; and MAP did not vary in the first case and decreased from 123 to 101 mmHg in the second. Over the following 24 months, proteinuria was <1 g/24 h in both cases and GFR increased in one patient by 2 ml/min and did not vary in the other patient. In this period, MAP and protein intake remained unvaried.

The data of Rossing et al. confirm that in the short term, complete blockade of the RAS system produces an antiproteinuric effect and affords a greater renoprotective effect since proteinuria is an important risk factor for kidney disease progression (4). Although our data can only be considered as observational findings, they do allow us to intuit that this renoprotective effect, due to the dual blockade of the RAS, will be increased when treatment is prolonged to medium or long term. Moreover, the observed decrease in total cholesterol levels would contribute to reducing both the progression of the nephropathy, since elevated serum cholesterol levels act as an independent promoter of progression in diabetic nephropathy (5), and the high cardiovascular risk seen in this type of patient.

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## Effect of 2-Week Treatment With Pirenzepine on Fasting and Postprandial Glucose Concentrations in Individuals With Type 2 Diabetes

**A**cute cholinergic muscarinic blockade with pirenzepine (PIR) induces dose-related reductions in plasma glucose (PG) and plasma insulin (PI) after mixed meals in normal individuals and in type 2 diabetic and polycystic ovary syndrome patients (1–2). Altogether, 24 patients with type 2 diabetes (12 male and 12 female) with a fasting plasma glucose  $\geq 10 \text{ mmol/l}$  were studied for the effects of PIR (50 mg b.i.d.) given for 2 weeks on fasting and postprandial plasma glucose and insulin concentrations. Patients on ACE inhibitors or thiazide diuretics were excluded.

Oral hypoglycemics were stopped at least 2 weeks before treating the patients with either PIR or placebo in a randomized, cross-over, double-blind fashion with a 2-week washout period between the two treatment arms. At the beginning (day 1) and end (day 14) of each treatment period, blood was sampled over 12 h, during which time the patients received three standard meals. The scheduling for blood sampling, meals, and PIR administration is illustrated in Fig. 1. The primary outcome measure for the study was the difference between PIR- and placebo-treated fasting plasma glucose on day 14 of the study. Statistical analysis was performed using the paired Student's *t* test.

There was a significant reduction in the fasting PG levels on day 14 (PIR) com-

pared with day 14 (placebo) (mean reduction  $\pm$  SD,  $1.2 \pm 1.6 \text{ mmol/l}$ , 95% CI 0.5–1.9,  $P = 0.002$  by paired Student's *t* test) (Fig. 1). There were trends for reduction in the glucose area under the curve and highest postprandial peaks for day 14 (PIR) compared with day 14 (placebo), but the results did not reach statistical significance. There was no significant difference in fasting plasma insulin levels.

The mechanism through which PIR produces its effect on PG is unknown. Possible hypotheses are the obliteration of an increased hypothalamic cholinergic tone responsible for increased 24-h growth hormone (GH) secretion, particularly suppression of nocturnal GH secretion, which we and others (3,4) have previously demonstrated. Another possibility is the effect of PIR on gastrointestinal hormones through its anticholinergic effect, which may affect PG levels indirectly (5).

In conclusion, PIR caused a statistically significant reduction in fasting PG levels compared with placebo after 2 weeks of therapy, but there was no corresponding increase in insulin levels. PIR may be an effective drug in the treatment of type 2 diabetes.

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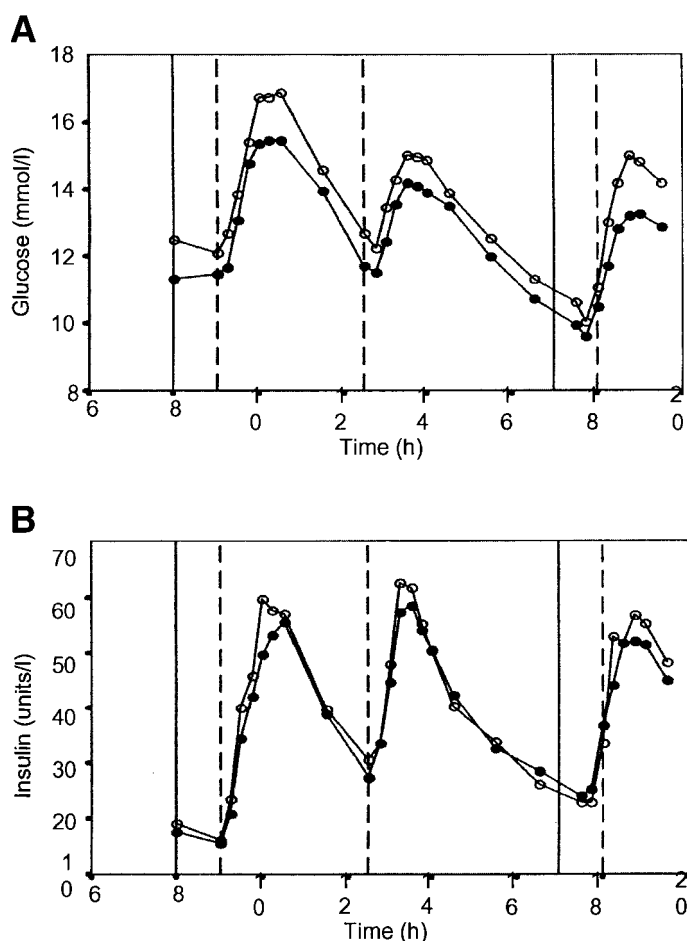
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**Figure 1**—Day curve of plasma glucose (A) and insulin (B) concentrations after 14 days of PIR (50 mg b.i.d.) (●) and placebo (○). The vertical continuous lines represent the time when pirenzepine was administered and the interrupted lines represent the times at which breakfast, lunch, and dinner were given.

ciated with an increased risk of cardiovascular disease and an increased mortality (3,4).

It is well known that insulin resistance is aggravated by chronic hyperglycemia. This hyperglycemia-induced insulin resistance is reversed by treatment, resulting in a prolonged period of euglycemia (5).

Although in animal studies evidence was found that hyperinsulinemia and/or insulin resistance increased total homocysteine levels (6), in humans the relation between insulin resistance and total homocysteine levels is unclear, in both healthy subjects (7–12) and patients with type 2 diabetes (13–16). In patients with type 2 diabetes, a positive association between insulin resistance and total homocysteine levels was reported (13), but this finding was not reproduced (14). Furthermore, in patients with type 2 diabetes, a positive association between the degree of metabolic control and total homocysteine levels was reported (15,16).

We hypothesized that if insulin resistance is associated with elevated homocysteine levels, thereby providing another explanation for the increased cardiovascular disease in type 2 diabetes, then homocysteine levels will decrease after amelioration of hyperglycemia-induced insulin resistance and associated metabolic abnormalities.

Here, we report the results of an intervention study (17). Eight obese type 2 diabetic patients (aged  $53 \pm 13$  years,  $HbA_{1c}$   $12.0 \pm 1.7\%$ , BMI  $38 \pm 5.8$  kg/m<sup>2</sup>) with severe insulin resistance (subcutaneous insulin dose, four-dose insulin regimen  $1.92 \pm 0.66$  units  $\cdot$  kg<sup>-1</sup>  $\cdot$  day<sup>-1</sup>) were studied before and after a period of  $28 \pm 5$  days of intravenous insulin treatment. Intravenous insulin treatment resulted in euglycemia. Before and after intervention, insulin sensitivity was assessed by a hyperinsulinemic-euglycemic clamp. Fasting plasma total homocysteine,  $HbA_{1c}$ , and lipid concentrations were measured on the days of the clamps. Also, 24-h urine creatinine excretion was investigated before and after intervention.

Plasma total homocysteine levels were measured with a high-performance liquid chromatography method according to Fiskerstrand (18,19) with some modifications. Because all variables were normally distributed, statistical analyses of differences were performed by paired Student's *t* test.  $P < 0.05$  was considered

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Prinz RA, Baron JH, Welbourne RB: The effect of pirenzepine on meal-stimulated gastrointestinal hormones. *Scandinavian J Gastroenterol* 66:57–61, 1980

## Improved Insulin Sensitivity and Metabolic Control in Type 2 Diabetes Does Not Influence Plasma Homocysteine

Type 2 diabetes is characterized by both insulin resistance and an increased cardiovascular mortality (1). Elevated plasma total homocysteine levels are an independent risk factor for cardiovascular disease (2). Also, in patients with type 2 diabetes, elevated plasma total homocysteine levels are asso-



significant. Results are given as the mean  $\pm$  SD.

After a 4-week period of intravenous insulin treatment resulting in an euglycemic period of  $17 \pm 4$  days, insulin sensitivity improved since body glucose uptake, measured by clamp, increased ( $12.7 \pm 5.7$  before vs.  $22.4 \pm 8.8 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  after euglycemia,  $P < 0.0005$ ) and the intravenous insulin dose required for achieving and maintaining euglycemia decreased ( $1.7 \pm 0.9$  before vs.  $1.1 \pm 0.6 \text{ units} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  after euglycemia,  $P < 0.005$ ). Metabolic control improved;  $\text{HbA}_{1c}$  decreased substantially ( $12.0 \pm 1.7$  before vs.  $8.9 \pm 1.2\%$  after euglycemia,  $P < 0.0001$ ); total cholesterol decreased ( $5.40 \pm 1.09$  before vs.  $4.50 \pm 1.10 \text{ mmol/l}$  after euglycemia,  $P < 0.05$ ); and triglycerides tended to decrease ( $4.34 \pm 3.32$  before vs.  $1.91 \pm 0.69 \text{ mmol/l}$  after euglycemia,  $P = 0.06$ ). These results are described in detail elsewhere (17). Plasma total homocysteine levels were similar before and after improved insulin sensitivity (total homocysteine levels  $8.5 \pm 2.4$  before vs.  $9.2 \pm 3.5 \mu\text{mol/l}$  after euglycemia,  $P = 0.5$ ). Urine creatinine excretion was similar before and after euglycemia ( $15.0 \pm 4.8$  before vs.  $12 \pm 4.3 \mu\text{mol}/24 \text{ h}$  after euglycemia).

This intervention study shows that despite amelioration of hyperglycemia-induced insulin resistance and improved metabolic control, plasma total homocysteine concentrations did not decrease. Homocysteine levels, measured in these patients with type 2 diabetes, were actually within the normal range of healthy subjects (2).

Our study is the first intervention study to investigate the relation between insulin resistance and plasma total homocysteine levels. To date only cross-sectional studies have been performed (13–15), which are more susceptible for confounding by other metabolic factors. Also in contrast to previous reports (8,9,11,12,14,15), we studied insulin sensitivity by clamp, the gold standard.

We conclude that amelioration of hyperglycemia-induced insulin resistance does not change plasma total homocysteine levels. These data refute the hypothesis that homocysteine levels are influenced by insulin resistance and by the degree of metabolic control, at least in the patients we studied. Therefore, these results do not support elevated homocysteine levels as an explanation for the link

between insulin resistance and the increased risk of cardiovascular disease in type 2 diabetes.

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## The True Overall Mortality of Unselected Nephropathic Type 2 Diabetic Patients With Mild to Moderate Renal Insufficiency

**D**espite significant improvements in therapy over the last decade, the prognosis of nephropathic type 2 diabetic patients continues to be poor (1). In recent antihypertensive intervention studies performed in type 2 diabetic patients with overt nephropathy, the mean 5-year mortality rate was ~20%. In the irbesartan study published by Lewis et al. (2), the mean death rate after 54 months ranged from 15 (irbesartan group) to 16.3% (placebo group). Similar data were obtained in the losartan study published by Brenner et al. (3). These results might lead to false conclusions concerning the overall mortality of all unselected type 2 diabetic patients with overt nephropathy. In the irbesartan study, major excretion criteria were congestive heart failure (New York Heart Association [NYHA] class III or worse) and cardiovascular events within the last 3 months.

In accordance with the irbesartan study, in 1996 we recruited all nephropathic type 2 diabetic patients who were treated at that time in our hospital and/or in the outpatient care unit and who fulfilled the inclusion criteria of the study (serum creatinine 1.0–3.0 mg/dl in female and 1.3–3.0 mg/dl in male patients, as well as protein excretion  $\geq 1.0$  g/24-h urine), without considering the usual exclusion criteria. We recruited 46 nephropathic type 2 diabetic patients (aged  $61 \pm 8$  years, 26 women and 20 men, diabetes duration  $17 \pm 4$  years), and we prospectively studied their progression of diabetic nephropathy and the 5-year mortality rate.

Cardiovascular disease was present in 43%, while 28% had heart failure (NYHA

class III and higher). Of patients, 36% had exclusion criteria according to the irbesartan study. All patients underwent evaluations in our outpatient unit ( $n = 24$ ) or at the offices of their general physicians in at least 6-month intervals over an observation period of 5 years. The following parameters were routinely measured: creatinine in serum and 24-h urine, creatinine clearance (calculated), protein in 24-h urine, HbA<sub>1c</sub>, and blood pressure. The three end points of the study were doubling of serum creatinine levels, end-stage renal disease, or death. Patients were also divided into those with and without heart failure, and 5-year mortality rates were assessed in both groups.

Serum creatinine had doubled in 37% of patients over 60 months, compared with 22% within 54 months in the irbesartan study. The percentage of patients who reached end-stage renal disease was 26% in our patients and 16.7% in the irbesartan study. During the period of observation, mean HbA<sub>1c</sub> level in our patients was  $7.6 \pm 1.0\%$ , while their mean blood pressure was  $139 \pm 11/80 \pm 8$  mmHg. In the irbesartan study, the mean blood pressure at visits after baseline was similar, with 140/77 mmHg in the irbesartan group and 144/80 mmHg in the placebo group.

The overall 5-year mortality of all of our diabetic patients was 33%. Five-year mortality rates were 57% in patients with heart failure (NYHA III or IV) and 22% ( $P < 0.05$ ) in those without heart failure. In comparison with the data of the intervention studies, the mortality of our nephropathic type 2 diabetic patients without heart failure was similar to that of the selected patients in the irbesartan study. In contrast, survival was significantly lower in patients with heart failure, and as a consequence, the overall mortality of all unselected type 2 diabetic patients with overt nephropathy was also higher than that registered in the intervention studies. There were no significant differences at baseline visit in creatinine clearance ( $139 \pm 31$  vs.  $134 \pm 32$  ml  $\cdot$  min<sup>-1</sup>  $\cdot$  1.73 m<sup>-2</sup>) and urinary protein ( $2.4 \pm 1.3$  vs.  $2.3 \pm 1.2$  g/24 h) in the groups with and without chronic heart failure, respectively. The mean blood pressure during the observation period was approximately the same in both groups ( $140 \pm 12$  vs.  $138 \pm 11$  mmHg). Twenty-eight percent of the patients with and 25% of those without heart failure

reached end-stage renal disease. Our results are in agreement with those of Short (4), who found, at a mean follow-up of 5.3 years, a mortality rate of 37% in nephropathic type 2 diabetic patients compared with just 8% in a nonnephropathic group. Multivariate Cox regression analyses confirmed a fivefold excess risk for cardiovascular mortality in type 2 diabetic patients with overt nephropathy. Angiotensin II blockers have shown effective cardiovascular protection in type 2 diabetic patients (5).

In conclusion, the real mortality rate of all unselected type 2 diabetic patients with overt nephropathy and mild-to-moderate renal insufficiency is much higher than the mortality of nephropathic type 2 diabetic patients selected for antihypertensive studies. The difference between outcome in studies versus "real life" is mainly due to the fact that patients with heart failure are excluded from studies, but obviously present in a nonstudy situation.

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## HLA-DR-DQ Haplotype in Rapid-Onset Type 1 Diabetes in Japanese

Type 1 diabetes is characterized by the presence of insulinitis and autoantibodies, at least in the earlier course of the disease. It is well known that HLA locus confers strong genetic susceptibility to the disease. Imagawa et al. (1) described a unique subtype of type 1 diabetes that shows a rapid and fulminant onset of the disease but lacks evidence of insulinitis and autoantibodies. Although the etiology of this unique subtype of type 1 diabetes is unclear at present, Tanaka et al. (2) very recently reported that specific HLA-DQ genotypes may be associated with this subtype of type 1 diabetes. Here, we report clinical characteristics of subjects with type 1 diabetes of a rapid and fulminant onset who were collected from the registration of type 1 diabetes in our institution.

We identified 19 type 1 diabetic subjects showing an acute and abrupt onset of diabetes (duration of hyperglycemic symptoms  $6 \pm 3$  days [mean  $\pm$  SD], range 1–10) accompanied by low HbA<sub>1c</sub> levels ( $6.8 \pm 0.9\%$ , 5.4–8.2). The subjects consisted of 11 men and 8 women aged  $38 \pm 17$  years (range 16–66) with BMI of  $22.2 \pm 3.2$  kg/m<sup>2</sup> (17.1–27.7). Family history of diabetes (possibly type 2 diabetes) was recorded in three subjects (16%). Among eight women, four manifested diabetes during gestation ( $n = 2$ ) or just after delivery ( $n = 2$ ). History of preceding upper respiratory infection was noted in nine subjects (47%). Despite short duration of subjective symptoms and low HbA<sub>1c</sub> levels, their initial concentrations of plasma glucose was as high as  $34.1 \pm 13.2$  mmol/l (range 13.6–72.4), indicating a fulminant onset of diabetes. In fact, 14 subjects showed diabetic ketoacidosis at their initial presentation (arterial pH  $7.14 \pm 0.17$ , 6.80–7.34), and the remaining five had marked ketosis and hyperglycemia. GAD autoantibodies,

measured by commercial RIA kits (Cosmic Corporation, Tokyo) (3), were negative in 18 of the 19 subjects ( $<1.5$  units/ml), but a single subject showed a weakly positive level (5.6 units/ml). Insulinoma-associated protein 2 (IA2) autoantibody and anti-thyroperoxidase and -thyroglobulin antibodies were negative in all subjects. Fasting serum level and urinary excretion of C-peptide were decreased to  $0.10 \pm 0.07$  nmol/l (0–0.26) and  $2.2 \pm 2.1$   $\mu$ mol/day (0.2–6.9), respectively, and they did not show any signs of remission of insulin dependency during the observation period.

As for HLA status, HLA-DRB1-DQB1 genotypes were determined and compared with those in autoantibody (GAD and/or IA2 autoantibodies)-positive subjects with significant type 1A diabetes ( $n = 87$ ) derived from our registration. Frequency of HLA-DRB1\*0405-DQB1\*0401 haplotype in a homozygous manner was significantly higher in the rapid-onset group (5 of 19, 26%) than the type 1A group (4 of 87, 5%,  $P = 0.009$  by Fisher's exact probability). The frequency was also far higher than that of nondiabetic Japanese subjects reported in the literature (1 of 84,  $P < 0.001$  [2] and 6 of 157,  $P = 0.003$  [4]). Frequency of other susceptible haplotypes (DRB1\*0405-DQB1\*0401 in a heterozygous manner and DRB1\*0901-DQB1\*0303 in a homozygous manner) (4) did not differ between the rapid-onset group and type 1A group (data not shown).

In our experience, homozygous HLA-DRB1\*0405-DQB1\*0401 haplotype confers genetic susceptibility to rapid-onset type 1 diabetes in Japanese, consistent with the recent report by Tanaka et al. (2). Frequency of GAD autoantibodies was actually low in this subgroup of type 1 diabetes (1,2), but a single subject showed a positive result at the onset of diabetes. There was also a case report in which GAD autoantibodies turned positive 1 year after the fulminant onset of type 1 diabetes (5). Tanaka et al. (6) also demonstrated presence of insulinitis in a case of rapid-onset type 1 diabetes. Collectively, it is suggested that rapid-onset type 1 diabetes could share autoimmune etiology with type 1A diabetes (7). Of interest is that half of the female patients developed diabetes during or just after pregnancy. There have been many studies reporting altered immune response during pregnancy (8,9), and pregnancy is considered

to be a precipitating factor for type 1 diabetes (10). We hypothesize that altered immune response to unknown stimulus may be causative of rapid and aggressive  $\beta$ -cell loss, together with the genetic susceptibility, at least in part, determined by HLA-DR-DQ haplotypes.

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## Sex, Diabetes, and Stroke After Carotid Endarterectomy

While diabetes is associated with increased risk of perioperative stroke among subjects who undergo carotid endarterectomy (1–3), women and minorities have been generally underrepresented in such trials (4). The North American Symptomatic Carotid Endarterectomy Trial (NASCET) was a randomized study of 2,885 eligible patients with carotid stenosis <70% who received either medical ( $n = 1,449$ ) or surgical therapy ( $n = 1,436$  patients) (3). The median study age was 66 years, and 93% of study subjects were white. Approximately 30% of NASCET subjects were female, and ~22% had a history of diabetes, which was almost always type 2 diabetes (3). The study showed that endarterectomy safely and efficaciously reduced the risk of stroke in symptomatic patients with higher-grade carotid arterial stenosis when compared with medical therapy (3). Multivariate analysis indicated an increased risk of perioperative stroke in subjects with a history of diabetes (relative risk [RR] 2.0, 95% CI 1.2–3.1) (3). Because NASCET had a relatively high proportion of both female and diabetic subjects, we addressed the hypothesis that there would be differences between diabetic men and women with respect to the risk of peri- and postoperative complications. Subjects were subdivided by surgical or medical therapy and then subdivided again by sex and diabetes history. End point data regarding strokes and deaths were collected.

Diabetic men had a significantly in-

creased risk of stroke or death 30 days postendarterectomy compared with nondiabetic men (RR 2.3, 95% CI 1.4–3.7), with no difference between diabetic and nondiabetic women (1.1, 0.5–2.4). Furthermore, diabetic men had a significantly increased risk of stroke 3 years postendarterectomy compared with nondiabetic men (RR 1.9, 95% CI 1.3–2.8), again with no difference between diabetic and nondiabetic women (1.0, 95% CI 0.6–1.9). However, at 3 years postrandomization, diabetic men who received medical treatment had no increased risk of stroke compared with nondiabetic men (RR 0.7, 0.5–1.1). In contrast, at 3 years postrandomization, diabetic women who received medical treatment had a significantly increased risk of stroke compared with nondiabetic women (1.7, 1.1–2.8).

The results support the emerging impression of sex-related differences among diabetic subjects with respect to vascular disease (5,6). Perioperative neurological morbidity of carotid endarterectomy is mainly due to embolization at the time of clamping or clamp release, ischemia during clamping of extracranial arteries, and intracerebral hemorrhage (7). There has been a steady decrease in postoperative morbidity among patients who undergo carotid endarterectomy as a result of both improved surgical technique and patient selection (7). Baseline clinical attributes are also helpful predictors of postoperative mortality and morbidity from stroke (3). Our analysis suggests that among diabetic subjects who undergo carotid endarterectomy, there may be sex-related differences in postoperative neurological morbidity. As with any post hoc subgroup analysis, the results should be interpreted cautiously and must be replicated in other studies. However, if these findings are confirmed, it might be appropriate to consider sex and diabetes status in strategies to optimize the management of patients with carotid disease (8).

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## The Diabetes Empowerment Scale—Short Form (DES-SF)

In 2000 we developed the Diabetes Empowerment Scale (DES) to measure the psychosocial self-efficacy of people with diabetes. The original questionnaire contained 37 items representing eight conceptual dimensions (i.e., assessing the need for change, developing a plan, overcoming barriers, asking for support, supporting oneself, coping with emotion, motivating oneself, and making diabetes care choices appropriate for one's priorities and circumstances). Using factor analyses the questionnaire was reduced to the current 28-item DES ( $\alpha = 0.96$ ) con-



taining three subscales (1). The three subscales are: 1) managing the psychosocial aspects of diabetes with 9 items ( $\alpha = 0.93$ ), 2) assessing dissatisfaction and readiness to change with 9 items ( $\alpha = 0.81$ ), and 3) setting and achieving goals with 10 items ( $\alpha = 0.91$ ). In addition to providing an overall assessment of diabetes-related psychosocial self-efficacy, the three subscales of the DES allow for an examination of its underlying components.

To allow for a brief overall assessment of diabetes-related psychosocial self-efficacy, we developed an eight-item short form of the DES (the DES-SF). The DES-SF was created by choosing the item from the remaining 28 items with highest item to subscale correlation from each of the original eight conceptual domains. The reliability of the DES-SF using the original dataset was  $\alpha = 0.85$ . We have subsequently administered the DES-SF to 229 subjects in a new study. The reliability of the DES-SF using the data from the new sample was  $\alpha = 0.84$ . The content validity of the DES-SF was supported in the new study by the fact that both DES-SF scores and HbA<sub>1c</sub> levels changed in a positive direction after the 229 subjects completed a 6-week problem-based patient education program (2). The change in DES-SF scores and HbA<sub>1c</sub> levels were not correlated, suggesting that these two measures vary independently.

These data provide preliminary evidence that the DES-SF is a valid and reliable measure of overall diabetes-related psychosocial self-efficacy. The DES and the DES-SF, scoring information, and permission to use them can be downloaded from the Michigan Diabetes Research and Training Center web site at: [www.med.umich.edu/mdrtc](http://www.med.umich.edu/mdrtc).

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## Increased Oxidative Stress Is Associated With Elevated Plasma Levels of Adrenomedullin in Hypertensive Patients With Type 2 Diabetes

**P**revious studies have demonstrated that oxidative stress is associated not only with hyperglycemia and hypertension but also with the metabolic syndrome and cardiovascular disease (1–3).

Adrenomedullin (AM) is a novel vasorelaxant peptide isolated from human pheochromocytoma (4,5). Oxidative stress enhances AM production from endothelium and vascular smooth muscle cells in vitro (6,7). However, whether oxidative stress is associated with circulating levels of AM in vivo is unknown. In the present study, we evaluated the relationship between the plasma levels of 8-epi-prostaglandinF2 $\alpha$  (8-epi-PGF2 $\alpha$ ; currently regarded as the most reliable marker for the assessment of oxidative stress in humans) (8,9) and AM in normal subjects and hypertensive patients with type 2 diabetes.

This study comprised 17 hypertensive patients with type 2 diabetes (15 men and 2 women, age  $47.3 \pm 3.0$  years [mean  $\pm$  SE], BMI  $23.2 \pm 0.8$  kg/m<sup>2</sup>, fasting plasma glucose  $8.2 \pm 0.5$  mmol/L, HbA<sub>1c</sub>  $9.9 \pm 0.5\%$ , fasting serum insulin  $30.6 \pm 3.0$  pmol/L, systolic blood pressure  $150.2 \pm 3.4$  mmHg, and diastolic blood pressure  $76.5 \pm 2.2$  mmHg) and 18 normal subjects (17 men and 1 woman, age  $43.0 \pm 1.9$  years, BMI  $23.8 \pm 0.4$  kg/m<sup>2</sup>, fasting plasma glucose  $5.1 \pm 0.2$  mmol/L, fasting serum insulin  $31.2 \pm$

$3.6$  pmol/L, systolic blood pressure  $126.8 \pm 2.2$  mmHg, and diastolic blood pressure  $79.8 \pm 1.6$  mmHg). All subjects were nonsmokers. Hypertensive patients with type 2 diabetes were diagnosed at a local clinic  $3.0 \pm 0.2$  years before the beginning of this study. All patients were treated with diet (1,440–1,720 kcal/day) and sodium restriction 304 mmol/day) and exercise (walking 10,000 steps/day); none of them were receiving any kind of drug. Their blood glucose and blood pressure were in good control (HbA<sub>1c</sub>  $\leq 6.5\%$ , systolic blood pressure  $< 140$  mmHg, and diastolic blood pressure  $< 90$  mmHg) during the initial several months. However, thereafter the blood glucose and blood pressure of the patients gradually increased (HbA<sub>1c</sub>  $\geq 8\%$ , systolic blood pressure  $\geq 140$  mmHg, and diastolic blood pressure  $\geq 90$  mmHg) because of overeating and inactivity. They were referred to our clinical department for control of hyperglycemia and hypertension. None of them had evidence of micro- or macroangiopathy. Informed consent was obtained from all subjects before the beginning of the study. Plasma levels of free 8-epi-PGF2 $\alpha$  were measured using a commercially available enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI). The detection limit of this assay was 1.5 pg/ml, and the intra- and interassay coefficients of variation were 7.5 and 9.2%, respectively. AM in plasma samples was measured using a commercially available immunoradiometric assay kit (Shionogi Pharmaceuticals, Osaka, Japan). The detection limit of this assay was 2 fmol/ml, and the intra- and interassay coefficients of variation were 7.0 and 6.9%. Serum insulin was measured using an immunoradiometric assay kit (Insulin Riabead II kit; Dainabot, Tokyo). The intra- and interassay coefficients of variation of the assay were 1.9 and 2.0%. In addition, we measured blood pressure in supine position after a 5-min rest.

Both plasma levels of 8-epi-PGF2 $\alpha$  and AM were significantly increased in hypertensive patients with type 2 diabetes compared with normal subjects (8-epi-PGF2 $\alpha$   $48.6 \pm 8.6$  vs.  $11.9 \pm 1.3$  pg/ml,  $P < 0.05$ ; AM  $14.8 \pm 0.7$  vs.  $12.4 \pm 0.2$  fmol/ml,  $P < 0.02$ ). The plasma levels of 8-epi-PGF2 $\alpha$  were proportionally correlated with AM ( $r = 0.696$ ,  $P < 0.01$ ) in only hypertensive patients with type 2 diabetes. Significant positive correlations were observed between plasma levels of

8-epi-PGF2 $\alpha$  ( $r = 0.540$ ,  $P < 0.05$ ) or AM ( $r = 0.875$ ,  $P < 0.001$ ) and systolic blood pressure in patients with type 2 diabetes.

This is the first report that demonstrated a relationship between oxidative stress and AM in vivo. The mechanism by which plasma levels of 8-epi-PGF2 $\alpha$  correlate with AM and the cellular source of AM remain unknown. Previous studies have shown that oxidative stress stimulates secretion of AM from endothelium and vascular smooth muscle cells and that AM mRNA expression is increased by activation of the nuclear factor- $\kappa$ B pathway (6,7). Increased AM secretion from endothelium and vascular smooth muscle may influence the plasma levels of AM. On the other hand, Shimomura et al. (10) reported that endogenous AM may protect from organ damage by inhibiting oxidative stress production. Increased AM levels may compensate for oxidative stress-induced vasoconstriction and thus may play a protective role against organ injury. Adrenal medulla and pancreatic islets may also be a source of AM (4,11). Further study is needed to clarify the source of plasma AM and the mechanism of correlation between oxidative stress and AM.

In conclusion, there was a significant positive correlation between increased oxidative stress and elevated plasma levels of AM in hypertensive patients with type 2 diabetes. Enhanced oxidative stress may regulate the plasma levels of AM in hypertensive patients with type 2 diabetes.

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## Renin-Angiotensin System Gene Polymorphisms and Retinopathy in Chinese Patients With Type 2 Diabetes

Diabetic eye disease is the leading cause of new cases of blindness in many developed countries, for which macular edema and proliferative retinopathy are major causes of loss of vision (1). Genetic studies have previously suggested that diabetic retinopathy is partly determined by genetic factors (2). Angiotensin II, produced locally within the retina, has potent hemodynamic and growth-promoting effects and stimulates ocular neovascularization (3,4). The presence of retinopathy is associated with an activated renin-angiotensin system (RAS) (5), and inhibition of the RAS significantly reduced the progression of retinopathy in nonhypertensive patients with type 1 diabetes (6). The ACE gene insertion/deletion (I/D) polymorphism has been reported to be associated with retinopathy in type 1 diabetic subjects, but the findings are heterogeneous (7,8). Only limited data are available describing the angiotensinogen (AGT) M235T and the angiotensin II type 1 receptor (AT<sub>1</sub>R) A1166C polymorphisms and retinopathy (9,10).

In this study, we examined the associations between these three polymorphisms and diabetic retinopathy in 827 Chinese type 2 diabetic patients (based on 1985 World Health Organization criteria). All subjects gave written informed consent and were of Han Chinese origin. They were examined by the physicians or ophthalmologists through dilated pupils. Retinopathy was considered to be present if there was one or more areas of hemorrhages, microaneurysms, cotton wool spots, and/or laser coagulation scars related to diabetic retinopathy or history of vitrectomy (11). The RAS genotype and allele frequencies were identified using PCR/restriction fragment-length polymorphism protocols (12) in 326 patients with retinopathy and 501 diabetic patients without retinopathy who were matched for age ( $59.8 \pm 11.4$  vs.  $60.4 \pm$

**Table 1—Genotype distributions for the ACE I/D, AGT M235T, and angiotensin type 1 receptor A1166C gene polymorphisms in type 2 diabetic patients without and with retinopathy**

Genotypes	No retinopathy	Retinopathy (total)	Nonproliferative	Advanced retinopathy (pre, proliferative, advanced)
n	501	326	225	101
ACE DD/ID/II	11.6/42.3/46.1	12.3/39.6/48.2	11.4/37.8/50.7	14.9/42.6/42.6
AGT TT/MT/MM	69.7/28.5/1.7	74.2/24.0/1.8	75.0/23.8/1.2	77.0/20.7/2.3
AT <sub>1</sub> R AC/AA	9.2/90.8	6.6/93.4	8.1/91.9	6.5/93.5

No significant differences were identified between the groups.

9.3 years), sex (44.2 female vs. 39.3% male), disease duration (6.3 [range 5.6–7.0] vs. 6.0 years [5.6–6.3]), and age of onset of diabetes ( $53.2 \pm 9.7$  vs.  $51.9 \pm 12.4$  years). Of those with retinopathy, 66.6% had nonproliferative, 8.3% preproliferative, and 8.9% proliferative disease and 16.2% had advanced eye disease. Of the patients, 30.1% with retinopathy had received laser therapy in at least one eye, with 18.7, 29.2, 53.8, and 69.4% being treated in the groups with increasing severity of retinopathy.

The allele frequencies were 32.7 and 32.1% for the ACE D allele, 16.1 and 13.8% for the AGT M allele, and 4.6 and 3.4% for the AT<sub>1</sub>R C allele in those without and with retinopathy, respectively. The genotype distributions for these gene polymorphisms are described in Table 1. No differences were identified in the genotype or allele frequencies between the groups either for the dichotomous classification of retinopathy or when the severity of the retinopathy was graded.

The ACE D allele has been associated with increasing ACE levels (12). Subjects with the angiotensinogen M235T polymorphism TT genotype have increased serum AGT levels, while the AT<sub>1</sub>R 1166C allele is associated with enhanced responsiveness to angiotensin II (13). The ACE DD genotype was found to be associated with the presence of proliferative retinopathy in a relatively small case-control study of type 1 diabetic subjects (7). In the current study, the analysis comparing age-, sex-, and diabetes duration-matched subjects found no evidence of associations between the distribution of genotypes or alleles of these RAS gene polymorphisms and retinopathy. Our data support the majority of the literature regarding the ACE I/D gene polymorphism, which has reported no significant association with retinopathy in diabetic patients (7,8).

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## Prevalence of Type 1 Diabetes-Related Autoantibodies in Adults With Celiac Disease

Studies have shown a 4% prevalence of celiac disease in type 1 diabetic patients. Few data, however, are available on the prevalence of type 1 diabetes-related antibodies in patients with celiac disease, with studies being limited by the recruitment of low numbers of children only (1,2). We have assessed prevalence of type 1 diabetes-related autoantibodies (islet cell antibody [ICA], GAD antibody [GADA], and antibodies to the protein tyrosine phosphatase-related IA-2 [anti-IA2]) in a cohort of 378 nondiabetic adults with celiac disease (89 untreated and 289 treated with a gluten-free diet), aged  $33.9 \pm 14.7$  years (range 15.7–81.3), who were cared for at our outpatient clinic. Known duration of glu-



ten withdrawal was <5 years in 146 (48.2%), 5–9 years in 54 (17.8%), and >9 years in 103 (34.0%) patients. GADAs were measured by a radioligand assay using human recombinant GAD 65 as antigen (Medipan Diagnostica, Selchow, Germany), ICAs by indirect immunofluorescence on frozen sections of human blood group 0 pancreas with fluorescein isothiocyanate-conjugated rabbit antibodies, and anti-IA2 by a radioligand assay using highly purified human recombinant IA2 labeled with  $^{125}\text{I}$  (Medipan Diagnostica).

Of 289 treated nondiabetic patients, 26 had type 1 diabetes-related autoantibodies, giving a prevalence of 9.0% (95% CI 6.7–12.3). All of them, however, showed no more than one marker positivity (2.8% ICA, 3.1% GADA, 3.1% anti-IA2). Untreated celiac patients had a similar prevalence (10.0%,  $P = 0.75$ ). In linear regression analysis, levels of anti-IA2 were linearly associated with duration of celiac disease ( $\beta = 0.002$ ,  $P = 0.05$ ). In logistic regression analysis, duration of gluten withdrawal was independently associated with a prevalence of type 1 diabetes-related autoantibodies. With respect to duration <5 years, a four-fold increased risk in patients with duration >9 years was found (95% CI 1.51–10.6), even after adjustment for age, sex, presence of other autoimmune diseases, and compliance to diet. In a mean follow-up time of  $3.0 \pm 1.3$  years (range 1.3–6.5), however, no incident case of type 1 diabetes was diagnosed in either patients with (79.0 person-years) or without (782.7 person-years) autoimmune markers.

In conclusion, this study shows that prevalence of type 1 diabetes-related autoantibodies in adults with celiac disease is high (9.0%), even after dietary gluten withdrawal, and that it increases over time but is associated with low risk of progression to diabetes. These findings are consistent with a role of common genetic susceptibility of both diseases, such as factors involved in intestinal permeability. Case-control studies show association between various dietary factors and risk of type 1 diabetes (3). Dietary gluten could act as modifier rather than determinant of type 1 diabetes, facilitating the progression of other dietary factors to the lamina propria, where they activate the autoimmune response against  $\beta$ -cells.

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## Glycemic Relapse After an Intensive Outpatient Intervention for Type 2 Diabetes

The effectiveness of interventions that reduce hyperglycemia in patients with diabetes (1,2) is limited by the tendency for glycemic relapse after the intervention ends. We sought to characterize the occurrence of glycemic relapse after initial improvement and to describe predictors of relapse.

Glycemic relapse was evaluated in 265 consecutive patients with type 2 diabetes who participated in a 3-month intensive outpatient intervention and were followed at least 1 year after completion of the intervention. Details of the intervention have been previously described (3). All had  $\text{HbA}_{1c} > 8\%$  before the intervention and had achieved marginal glycemic control (nadir  $\text{HbA}_{1c} < 8\%$ ) after the intervention. Glycemic relapse was defined as a subsequent  $\text{HbA}_{1c} \geq 8\%$  and an ab-

solute increase of at least 1% above the postintervention nadir  $\text{HbA}_{1c}$ . All data were analyzed using SAS 8.12 (SAS Institute, Cary, NC).

The mean  $\pm$  SD age was  $56 \pm 13.4$  years; 54% were women; 71% were Caucasian; and 27% were African American. The mean duration of diabetes was  $6.4 \pm 8.4$  years. Mean BMI was  $32.2 \pm 8.6$  kg/m $^2$ . The mean  $\text{HbA}_{1c}$  before intervention was  $10.1 \pm 1.7\%$ . The mean  $\text{HbA}_{1c}$  nadir after intervention was  $6.8 \pm 0.7\%$ . Twenty-five percent were receiving insulin therapy before the intervention, and an additional 25% initiated insulin during the program.

The cumulative incidence of relapse at 1 year was 25%. The initiation of insulin therapy during the intervention was the only identifiable independent predictor of relapse (hazard ratio 1.96, 95% CI 1.02–3.74). Female sex, African-American race, duration of diabetes, lack of weight loss during the intervention period, and the levels of  $\text{HbA}_{1c}$  before the intervention and at nadir after the intervention increased the risk of relapse in univariate analysis, but these associations were not statistically significant in multivariate modeling. For those patients who relapsed, the median time to relapse was 9 months. Kaplan-Meier plots estimated that 50% of the population would relapse by 30.3 months.

Twenty-five percent of type 2 patients who attained satisfactory glycemic control after an intensive outpatient intervention relapsed within a year. Patients who initiated insulin therapy during the intervention had an almost double risk of relapse. Future studies should characterize social and behavioral variables as well as the frequency and type of insulin in order to better understand the relapse process. In the interim, this high-risk subgroup should receive priority for continuation of intensive care or other relapse preventive measures.

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## Gestational Glucose Intolerance in Multiple Pregnancy

**T**win pregnancies have been associated with adverse obstetric and neonatal outcomes. The incidence of respiratory distress syndrome in the newborns of multiple pregnancy patients is probably related to the higher incidence of preterm delivery. However, the complications of twin pregnancies are not limited to infants; miscarriages, preeclampsia, anemia, placenta previa, polyhydramnios, and premature rupture of membranes are more frequent than in singleton pregnancy. There have been a few reports that test the hypothesis that multiple gestation could also be a risk factor for gestational diabetes (1-6). These studies utilized various criteria for diagnosing gestational diabetes (World Health Organization [1], National Diabetes Data Group [2-4], and Carpenter and Coustan [7]) and did not report the percentage of the positive glucose challenge test for each group.

To determine the prevalence of gesta-

tional diabetes (using the Carpenter and Coustan criteria) in singleton and twin pregnancies in a cohort population of women who had had screening for gestational diabetes from January 1990 to January 2000, we started a retrospective study on 2,554 pregnant women screened for gestational diabetes in the Department of Obstetrics and Gynecology of our University Hospital. Of these, 70 were multiple pregnancies. A 50-g oral glucose challenge test was performed between 24 and 28 weeks' gestation, and a 1-h value  $\geq 135$  mg/dl was considered positive and followed by a 100-g oral glucose tolerance test. The results were interpreted according to the Carpenter and Coustan threshold values (95, 180, 155, and 140 mg/dl) at fasting and after 1, 2, and 3 h, respectively. If two thresholds were met or exceeded, then the diagnosis of gestational diabetes was made. Age, familial or personal anamnestic factors, parity, and BMI at first visit, all considered risk factors for GDM, were recorded in our database. A positive glucose challenge test occurred in 520 of 2,554 pregnant women (20.3%). The test was positive in 22 of 70 (31.4%) multiple pregnancies and 498 of 2,484 (20.0%) singleton pregnancies ( $P = 0.029$ ). The rates of GDM were 80 of 2,484 (3.6%) in singleton and 4 of 70 (5.7%) in twin pregnancies ( $P = 0.416$ ). No statistically significant differences exist for the maternal risk factors for GDM between the two groups.

The decreased insulin sensitivity in pregnancy may be modified by several factors, such as diet, BMI, maternal age, and the placental mass, all of which may play a role affecting  $\beta$ -cell function and sensitivity to insulin. It has been suggested that in multiple pregnancies with two placentas or one that is larger, the incidence of gestational diabetes may be increased (3,4,6). Our data show that in our Sicilian population, twin pregnancy cannot be considered a proven risk factor for gestational diabetes. In fact, the differences (3.6 vs. 5.7%) are not statistically different. Our data showed that one-third of twin pregnancies (22 of 70, 31.4%) manifest a positive glucose challenge test, as compared with 20% of singleton pregnancies (498 of 2,484). It seems possible that with a larger sample size, the difference in incidence of gestational diabetes might have been statistically significant, although of questionable clinical signifi-

cance. Another hypothesis could be that the greater relative elevation of anti-insulin placental hormone levels in a twin gestation may precipitate a mild degree of glucose intolerance in a woman who would have been normal with a singleton pregnancy. If this were the case, the impact of such a mild degree of glucose intolerance remains undetermined.

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## Cytokine Milieu Tends Toward Inflammation in Type 2 Diabetes

It has been postulated that type 2 diabetes is a manifestation of the inflammatory host response (1). Increased inflammatory activity is believed to play a critical role in development of atherogenesis and to predispose established atherosclerotic plaques to rupture (2). Interleukin (IL)-8 is a potent chemoattractant (chemokine) and induces recruitment of neutrophils and T-cells into the subendothelial space, as well as adhesion of monocytes to endothelium (3). IL-18 is a potent proinflammatory cytokine reported to play a role in plaque destabilization and to predict cardiovascular death in patients with coronary artery disease (4). Adiponectin is an adipocyte-derived plasma protein (adipokine) that accumulates in the injured artery and has potential anti-atherogenic properties; male patients with hypoadiponectinemia present a two-fold increase in coronary artery disease prevalence (5).

We studied 30 patients (age  $42 \pm 4$  years, BMI  $26.9 \pm 1.2$  kg/m<sup>2</sup>, and HbA<sub>1c</sub>  $7.2 \pm 0.6\%$  [mean  $\pm$  SD]) with newly diagnosed type 2 diabetes (within 6 months of diagnosis) without hypertension or evidence of vascular complications treated only by diet. Thirty healthy subjects, matched for age, sex, and body weight, served as the control group. None of the subjects (diabetic and nondiabetic) were taking any drugs. Serum samples for IL-8, IL-18, and adiponectin were stored at  $-80^\circ\text{C}$  and measured in duplicate using enzyme-linked immunosorbent kits. All samples for a given patient were analyzed in the same series.

Circulating levels of IL-8 and -18 concentrations were higher in diabetic patients ( $13.7 \pm 2.8$  and  $205 \pm 39$  pg/ml, respectively) than in control subjects ( $8.7 \pm 1.9$  and  $120 \pm 25$  pg/ml,  $P < 0.05$ – $0.001$ ), while circulating adiponectin levels were lower ( $4.9 \pm 1.2$  vs.  $7.1 \pm 1.9$   $\mu\text{g/ml}$ ,  $P < 0.01$ ). There was a significant correlation between fasting glucose and IL-8 levels in diabetic patients ( $r = 0.31$ ,  $P < 0.05$ ); adiponectin concentrations were negatively correlated with fasting insulin levels ( $r = -0.43$ ,  $P < 0.01$ ).

Our study shows that circulating IL-8

concentrations are significantly higher and adiponectin levels are significantly lower in type 2 diabetic patients than matched control subjects. Previous studies reported similar findings (6,7). To our knowledge, this is the first demonstration that circulating IL-18 concentrations are increased in type 2 diabetic patients, as compared with age-, sex-, and body weight-matched nondiabetic subjects. In a prospective study of 1,229 subjects, including one-sixth of diabetic patients, with documented coronary artery disease, serum IL-18 concentration was identified as a strong independent predictor of future cardiovascular events (5). The correlation we found between IL-18 and glucose levels in diabetic patients and the evidence that acute hyperglycemia may increase circulating IL-18 levels in healthy subjects and IGT patients (8) suggest a role for this cytokine in plaque destabilization associated with stress hyperglycemia (9).

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## Lipoprotein Abnormalities in Human Genetic CD36 Deficiency Associated With Insulin Resistance and Abnormal Fatty Acid Metabolism

CD36 is an 88-kDa membranous protein with multiple relevant function that is widely expressed in human tissues (1). Because we and others (2) found human genetic deficiency of this molecule, we have elucidated the molecular bases and pathophysiology. We have reported that the human deficiency may be associated with insulin resistance (3) and abnormal dynamics of long-chain fatty acid (LCFA) (4). The aim of the present study was to further characterize lipid and lipoprotein metabolism in the human genetic CD36 deficiency, especially focusing on postprandial responses.

Table 1—Lipid and lipoprotein abnormalities at fasting and postprandial states in human genetic CD36 deficiency

	Group A			Group B					
	Case 1	Case 2	Control (n = 4)	Case 3	Case 4	Case 5	Case 6	Case 7	Control (n = 8)
Age (years)/sex (M/F)	34/M	32/M	33 ± 2 (4M)	58/M	65/F	54/F	54/F	54/M	55 ± 10 (3M/5F)
BMI (kg/m <sup>2</sup> )	22.9	32.0	22.4 ± 1.0	26.2	22.2	32.8	17.3	24.9	22.7 ± 2.3
WBGU (mg · kg <sup>-1</sup> · min <sup>-1</sup> )*	7.8	2.8	9.1 ± 0.5	2.67†	7.09†	5.49†	4.85†	5.29†	8.6 ± 0.5†
Fasting state									
Total cholesterol (mmol/l)	4.20	5.01	4.78 ± 0.38	5.81	6.18	5.97	5.45	5.84	5.10 ± 0.69
Triglyceride (mmol/l)	0.78	1.23	1.00 ± 0.17	2.73	1.43	1.77	1.28	3.41	1.20 ± 0.38
Midband in PAG disc electrophoresis‡	—	—		+	+	+	+	+	
IDL cholesterol (mmol/l)§	ND	ND		0.48	0.38	0.29	0.34	0.22	0.11 ± 0.03
Postprandial state									
AUC-TG (mmol/l 6 h)	5.3	8.8	8.8 ± 0.7	20.6	13.4	14.0	14.0	26.8	10.0 ± 2.9
AUC-Apo B48 (AU/ml 6 h)**	223	268	329 ± 107	866	671	941	551	541	360 ± 120

Data are means ± SD unless otherwise indicated. apo, apolipoprotein; AUC, area under the curve; PAG, polyacrylamide gel; WBGU, mean whole-body glucose uptake. \*Insulin resistance was determined by euglycemic clamp technique (ref. 3); †part of data from cases 3–7 were described previously (Ref. 3); ‡lipoproteins were analyzed by PAG electrophoresis. §IDL was separated by preparative ultracentrifugation (1.019 < d < 1.063 g/ml); ||postprandial hyperlipidemia was analyzed by the oral fat loading test with fat cream containing 30 g fat/m<sup>2</sup> body surface area; TG and apo B48 levels were measured at 0, 2, 4, and 6 h after loading, and AUCs were calculated; \*\*serum apo B48 levels were measured by the enzyme-linked immunosorbent assay we have developed.

Seven Japanese patients with type 1 CD36 deficiency (cases 1–7) were subjected. They were divided into the following two groups: group A, two younger patients we have recently identified (cases 1 and 2); and group B, patients >50 years of age (cases 3–7, reported in our previous paper [3]). For the analysis of each group, aged-matched healthy and nonobese volunteers were subjected as control subjects (Table 1). First, we confirmed that insulin resistance was also present in the two younger patients (group A).

At the fasting state, “midbands,” which imply the accumulation of remnant lipoproteins, were observed in group B but not in group A. Preparative ultracentrifugation confirmed an increase in intermediate-density lipoprotein cholesterol in the all of the CD36-deficient subjects in group B compared with the control subjects. The oral fat loading test demonstrated that the patients in group B presented postprandial hyperlipidemia with the accumulation of small intestine-derived lipids. Whereas the patients in group A did not show apparent abnormalities, though they did have insulin resistance.

The present study indicated that this monogenic disorder with the abnormal metabolism in LCFA and insulin resistance may cause the phenotypic expression of hyperlipidemia at both fasting and postprandial states. These data also suggest that the clinical manifestation is affected by the acquired factors, such as aging and dietary contents. We believe

that human genetic CD36 deficiency may be a monogenic form of “metabolic syndrome.” Further studies to elucidate molecular mechanism for the phenotypic expression in CD36-deficient states are under way in our laboratories.

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## The Relation Between Physical Activity and Metabolic Control in Type 2 Diabetes With <20 Years of Evolution

Obesity and reduced levels of physical activity are directly associated with the insulin resistance that characterizes type 2 diabetes (1,2). Physical activity is one of the cornerstones of type 2 diabetes management (3). Few



**Table 1—Demographic, comorbidity, lifestyle, biochemical, and somatometric characteristics by physical activity levels (quartiles) of type 2 diabetic patients who participated in the KAREN study**

	<51 kcal/day	51–148 kcal/day	149–264 kcal/day	>264 kcal/day	P
n	368	373	375	369	
Age (years)	62 ± 10	61 ± 10	61 ± 9	61 ± 10	NS
Women (%)	49.6	45.3	54.4	69.6	<0.001
Time with diabetes (years)	10.3 ± 6.4	9.9 ± 6.3	10.1 ± 6.4	10.3 ± 6.2	NS
Hypertension (%)	51.8	58.3	50.7	46.3	0.017
Current smokers (%)*	19.4	18.9	19.0	16.0	NS
Systolic BP (mmHg)	140 ± 17	139 ± 17	139 ± 16	139 ± 19	NS
Diastolic BP (mmHg)	80 ± 10	79 ± 9	79 ± 10	78 ± 11	NS
BMI (kg/m <sup>2</sup> )	28.8 ± 3.4	28.5 ± 3.5	28.1 ± 3.3	28.3 ± 3.3	0.035
Coronary heart disease (%)†	17.9	14.4	18.0	14.0	NS
Cerebrovascular disease (%)‡	8.5	6.4	6.6	5.1	NS
Glycemia (mg/dl)	9.5 ± 3.2	9.1 ± 2.9	9.1 ± 3.0	8.8 ± 2.7	<0.001
HbA <sub>1c</sub> (%)*	7.9 ± 1.6	7.6 ± 1.4	7.6 ± 1.4	7.4 ± 1.0	<0.001
Total cholesterol (mmol/l)	5.3 ± 1.0	5.4 ± 1.0	5.2 ± 1.0	5.1 ± 1.0	<0.001
HDL cholesterol (mmol/l)	1.2 ± 0.3	1.3 ± 0.3	1.2 ± 0.3	1.3 ± 0.4	0.014
LDL cholesterol (mmol/l)	3.4 ± 0.9	3.4 ± 0.9	3.2 ± 34.7	3.3 ± 0.9	NS
Triglycerides (mmol/l)	1.9 ± 1.8	1.7 ± 0.9	1.7 ± 1.1	1.5 ± 0.9	<0.001
Creatinine (μmol/l)	91.9 ± 31.8	85.7 ± 24.7	86.6 ± 26.5	88.4 ± 19.4	0.035
Good diet compliance (%)	37.1	35.1	49.2	52.2	<0.001
Daily alcohol consumption (g)	12.9 ± 12.8	12.5 ± 12.3	12.8 ± 13.3	12.5 ± 12.3	NS

Data are means ± SD and %. \*Smokers of at least one cigarette per day in average; †myocardial infarction or angina; ‡transient ischemic attack or stroke.

large studies have assessed the impact of intensity and quality of exercise on metabolic control in these patients, and the influence of age on the metabolic response to physical activity is still controversial (4). We conducted the Intervention on Key Cardiovascular Risk Factors in Type 2 Diabetes National Study (KAREN), a country-based case-control study nested in a survey of 1,485 consecutive type 2 diabetic patients treated by 208 endocrinologists across Spain to assess the impact of physical activity on metabolic control. Patient risk factors, history characteristics, medication and diet compliance, glycemia, fasting HbA<sub>1c</sub>, and lipids were determined.

Patient characteristics are presented in Table 1 by quartile of physical activity. After adjustment for age, sex, triglycerides, BMI, and diet compliance, patients in the lowest quartile of physical activity had an OR of 1.52 and a 95% CI of 1.11–2.08 for having an HbA<sub>1c</sub> >7.5%. The ORs for patients <65 years of age and in the lowest quartile of physical activity were 1.55 (95% CI 1.04–2.31) compared with the fourth, 1.64 (1.09–2.47) with the third, and 1.84 (1.22–2.75) with the second quartile. Patients >65 years of age and in the lowest quartile of physical activity had a similar adjusted risk com-

pared with the rest of quartiles (OR 1.14, 95% CI 0.70–1.85; 1.38, 0.84–2.26, and 0.87, 0.53–1.43, respectively). A significant reduction of 0.2% (95% CI –0.36 to –0.04) in HbA<sub>1c</sub> was observed for patients expending >50 kcal/day versus the first quartile; the reduction for patients ≤65 years of age was 0.27% (–0.49 to –0.06), but for patients >65 there was no significant difference.

These results suggest that physical activity produces better glycemic control and improved cardiovascular risk profile in diabetic patients, particularly in those <65 years of age.

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## Appendix

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## Education and Mortality in Type 2 Diabetes

Adults with diabetes tend to have higher age-adjusted mortality rates (1,2). Evidence from Britain (3) suggests that mortality in diabetic adults is inversely related to socioeconomic status. The importance of self-care and blood glucose control in adults with diabetes suggests that education may be an important factor in long-term health outcomes, a hypothesis that is also consistent with evidence in the health economics literature that education increases the efficiency of investment in health (4,5). The specific hypothesis tested here is that mortality in adults with diabetes is inversely related to education.

The data are taken from the National Health Interview Survey (NHIS) of 1989, including its diabetes supplement, with mortality status taken from the NHIS Multiple Cause of Death Public Use Data File, including deaths from 1989 to 1995. The statistical analysis is applied to those adults who self-reported that they had been diagnosed with diabetes, excluding those with apparent type 1 diabetes (age of onset <30 years, with insulin dependence, and a BMI <27.2 kg/m<sup>2</sup> for men and <26.9 for women). The resulting sample includes 2,387 adults with type 2 diabetes having an average age of 61.8 years.

Logistic regression analysis is used to explore the relation between mortality and educational attainment, family income (greater than or less than \$20,000 per year in 1989), age, sex, marital status, race, and duration of diabetes. The statistical analysis is carried out with the svylogit procedure in STATA software, version 7 (STATA, College Station, TX), using the NHIS sampling weights.

The primary finding is that mortality is inversely related to education for diabetic adults (OR [95% CI] 0.61 [0.40–0.93] for college graduation or higher and 0.78 [0.61–0.99] for high school graduation or higher). Mortality is not significantly related to family income, marital status, or race, but age, male sex, and duration of diabetes have the expected positive and statistically significant associations with mortality. The finding that education reduces the mortality risk of diabetic adults is consistent with the predictions

from health investment theory that education increases the efficiency of health investment. Education may also be a factor in the relatively poor health status and outcomes of adults with diabetes (6).

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## Stress Hyperglycemia, Inflammation, and Cardiovascular Events

One-third of all individuals with hyperglycemia admitted to an urban general hospital do not have a previous diagnosis of diabetes; in these pa-

tients, hyperglycemia is a risk factor for adverse outcomes during acute illness (1). For example, in patients presenting with acute myocardial infarction, glucose values in excess of 6.1–8.0 mmol/l were associated with a threefold increase in mortality and a higher risk of heart failure (2). Control of hyperglycemia with intensive insulin therapy during acute illness results in marked improvements of clinical outcomes (3).

Dandona et al. (4) put forward a working hypothesis linking in a feedback loop of glucose (and possibly other nutrients), insulin, and inflammation. According to this paradigm, hyperglycemia has a proinflammatory action that is normally restrained by the anti-inflammatory effect of insulin secreted in response to that stimulus. A likely corollary of this paradigm is that the proinflammatory effects of acute (stress) hyperglycemia may be implicated in the poor prognosis of hospitalized patients with or without diabetes (particularly death, disability after acute cardiovascular events, and infections). We have demonstrated that circulating levels of some proinflammatory cytokines are regulated by glucose levels in humans (5). Acute hyperglycemia (~15 mmol/l) induced by glucose clamping in normal subjects and lasting 5 h determines a significant increase of interleukin (IL)-6, IL-18, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) circulating levels. These effects were more sustained in patients with impaired glucose tolerance, as well as following consecutive pulses of intravenous glucose, and were annulled by glutathione, implicating an oxidative mechanism. Interestingly enough, these cytokines have been implicated in insulin resistance (TNF- $\alpha$  and IL-6), atherosclerotic plaque destabilization (IL-18), and future cardiovascular events (IL-6, IL-18, and TNF- $\alpha$ ). So, an increased oxidative stress may be a likely mechanism linking stress hyperglycemia to cardiovascular events via an increased cytokine production.

Free fatty acids (FFAs) also induce proinflammatory changes. During illness, stress increases the concentration of counterregulatory hormones (mainly glucagon, an epinephrine). Given this background, it is plausible that in the presence of high concentrations of both glucose and FFA, inflammation is more prominent. We tested this hypothesis by giving type 2 diabetic patients two different meals: a high-carbohydrate meal (high

glucose levels) and a high-fat meal (high glucose plus high FFA levels). In the latter condition, the serum concentrations of cytokines (TNF- $\alpha$  and IL-6) and adhesion molecules (intracellular adhesion molecule-1 and vascular cell adhesion molecule-1) were at the highest level (6).

In conclusion, high circulating concentrations of glucose and FFAs may explain, at least in part, the oxidative and inflammatory derangements during acute illness; insulin may exert its anti-inflammatory action by ameliorating glucose and lipid parameters. However, any additional anti-inflammatory effect insulin may have of its own is welcome. The importance is to recognize that treatment of even trivial hyperglycemia during acute illness is fundamental to improve survival. Because insulin is the best choice to normalize glucose control during stress, accepting the premise will inevitably bring increased insulin use in intensive care units.

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## Laughter Lowered the Increase in Postprandial Blood Glucose

Negative emotions such as anxiety, fear, and sorrow are known to be factors that elevate the blood glucose level (1). Conversely, positive emotions such as laughter have been reported to modify the levels of neuroendocrine factors involved in negative emotions (2,3) and to modulate immune function (3,4). However, there have been no studies on the effects of laughter on blood glucose level. The purpose of this study was to clarify changes in the blood glucose level after laughing episodes in patients with diabetes.

A 2-day experiment was performed in 19 patients with type 2 diabetes not receiving insulin therapy (16 men and 3 women, age  $63.4 \pm 1.3$  years, BMI  $23.5 \pm 0.7$  kg/m<sup>2</sup>, HbA<sub>1c</sub>  $7.2 \pm 0.1\%$  [means  $\pm$  SE]) and 5 healthy subjects (2 men and 3 women, age  $53.6 \pm 3.5$  years, BMI  $24.3 \pm 1.6$  kg/m<sup>2</sup>, HbA<sub>1c</sub>  $4.8 \pm 0.1\%$  [means  $\pm$  SE]). On both experimental days, they consumed the same 500-kcal meal (79.9 g carbohydrate, 21.0 g protein, 7.8 g fat, and 1.0 g fiber). On the first day, they attended a monotonous lecture (40 min) without humorous content. On the second day, as part of an audience of 1,000 people attending MANZAI (a Japanese cross-talk comedy) (40 min) in a civic hall, the subjects laughed. Blood glucose was measured from the fingertip by enzyme colorimetric assay using a blood glucose self-measurement apparatus. The subjects estimated their laughter level on a scale of

0–5, and most of them considered that they laughed well (level 4 or 5). Self-monitoring of blood glucose was performed before food intake (fasting blood glucose [FBG]) and 2 h after the meal was started (2-h postprandial blood glucose [PPBG]).

The results are presented as means  $\pm$  SE. In the patients, the mean 2-h PPBG was  $6.8 \pm 0.7$  mmol/l higher than the FBG after the lecture and  $4.3 \pm 0.8$  mmol/l higher after the comedy show. The difference in the mean increase between the lecture and comedy show was  $2.5 \pm 0.7$  mmol/l ( $P < 0.005$ ). In the healthy subjects, the mean increases were  $2.0 \pm 0.7$  and  $1.2 \pm 0.4$  mmol/l after the lecture and comedy show, respectively, and the difference was  $0.8 \pm 0.5$  mmol/l ( $P = 0.138$ ).

These results suggest a significant suppression of the increase in 2-h PPBG by comedy show in patients with diabetes, suggesting that laughter ameliorates the postprandial glucose excursion in the presence of insufficient insulin action. This favorable effect of laughter may include the acceleration of glucose utilization by the muscle motion during the comedy show. However, it is possible that positive emotions such as laughter acted on the neuroendocrine system and suppressed the elevation of blood glucose level.

In conclusion, the present study elucidates the inhibitory effect of laughter on the increase in PPBG and suggests the importance of daily opportunities for laughter in patients with diabetes.

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## Differential Levels of $\gamma$ -Glutamyl Transferase Activity and Apolipoprotein CIII in Men on Either Statin or Fibrate Therapy

High triglycerides and low HDL cholesterol are frequently associated with insulin resistance. Statins are potent hypocholesterolemic drugs, while fibrates are better at reducing triglycerides and enhancing HDL. However, little is known of their effects on other parameters potentially associated with insulin resistance, such as  $\gamma$ -glutamyl transferase (GGT) (1) and apolipoprotein CIII (2).

In this cross-sectional study, we have analyzed the biochemical profiles of 500 men from Southwestern France as a function of their hypolipidemic therapy. Comparison between untreated ( $n = 361$ ) and fibrate ( $n = 50$ )- and statin ( $n = 89$ )-treated subjects revealed no difference in their levels of total, LDL, and HDL cholesterol, apo B, apo AI, and apo E, after adjustment for age, BMI, alcohol intake, smoking, and physical activity. This suggests that both therapies are equally effective at normalizing classic lipid variables.

By contrast, apo CIII was higher in the statin group ( $34.3 \pm 13.9$  mg/l) than in both the fibrate ( $26.7 \pm 10.3$ ,  $P < 0.01$ ) and nontreated ( $28.7 \pm 10.9$  mg/l,  $P < 0.001$ ) groups after adjustment for con-

founders, including triglycerides. Similar differences were found for Lp B:CIII, a marker measuring apo CIII associated with apo B. These observations might indicate an additional beneficial effect of fibrates, considering that Lp B:CIII has been described as an independent predictor of coronary events, better than triglycerides (3).

Most interesting, GGT was two times lower for fibrates ( $36.7 \pm 24.4$  units/l) than for the two other groups ( $67.1 \pm 71.3$  units/l for statins,  $P < 0.01$  and  $58.1 \pm 51.7$  units/l in nontreated subjects,  $P < 0.01$ ), taking into consideration potential confounders, including alcohol intake and triglycerides. GGT is often elevated in obese subjects and has been associated with steatosis, which could be due to increased effects of insulin in the liver and could contribute to the development of systemic insulin resistance and hyperinsulinemia in obesity. GGT was reported as one of the independent predictors of the metabolic syndrome and type 2 diabetes (1,4). Moreover, GGT was detected in atheromatous plaques and has been shown to promote LDL oxidation by hydrolyzing glutathione into more potent iron reductants (5). Thus, fibrates may prove effective in the treatment of dyslipidemic diabetic or obese patients, since they should improve the hepatic suffering induced by the triglyceride load and also limit the potential proatherogenic effects of GGT.

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## COMMENTS AND RESPONSES

### IGF-1 and Macrovascular Complications of Diabetes

Alternative interpretations of recently published data

The hypothesis of a detrimental role for IGF-1 and other growth factors in the development of vascular disease (1) has not been consistently supported by recent studies (2,3). New evidence suggests that cellular senescence (4) and impaired vascular endothelial proliferation, adhesion, and incorporation play a pivotal role in the development of macrovascular disease (5). Indeed, recent data on large numbers suggest that higher IGF-1 bioavailability may protect against the onset of ischemic heart disease (6,7) and glucose intolerance (8) and, in type 2 diabetic patients, may offer improved metabolic control and prevent vascular complications (9,10). Other potential beneficial actions of IGF-1 in car-

diovascular physiology include increased nitric oxide synthesis and K<sup>+</sup> channel opening (11,12), and this may explain the impaired small-vessel function associated with low IGF-1 levels in patients with cardiovascular syndrome X (12). By counteracting oxidized LDL-induced cytotoxicity and vascular smooth muscle cell apoptosis, IGF-1 may protect against plaque instability and rupture (2). In patients with acute myocardial infarction, markedly reduced IGF-1 values are associated with a worse outcome (13,14), and recent data suggest that intramyocardial (15,16) or vascular (17) gene delivery of growth factors can improve symptoms and exercise capacity in patients with coronary or peripheral vascular disease.

We were therefore surprised by two recent studies proposing IGF-1 as a mediator of harmful vascular effects (18,19). The authors found an inverse association between IGF binding protein (IGFBP)-1 and carotid intimal-medial thickness ( $r = -0.135$ ,  $P = 0.041$ ) (18), macrovascular disease (19), and hypertension (19) in patients with type 2 diabetes. IGFBP-1 is a minor component of the system of IGFBPs 1–6, first discovered in the 1990s, and regulated by a complex group of proteases and phosphatases (20). As IGFBP-1 is generally assumed to inhibit IGF-1 bioavailability (21), the authors conclude that IGF-1 exerted harmful vascular effect (18,19).

We propose two different interpretations of the above data, not necessarily exclusive. First, the reduced IGFBP-1 concentration observed in situations of vascular disease (18,19) could result from activation of a compensatory mechanism leading to higher IGF-1 bioavailability. This interpretation is confirmed by the finding in hypertensive patients of higher IGF-1 levels than in control subjects (22), and of a positive association among IGF-1, insulin sensitivity, and preserved vasodilator capacity (23,24). Indeed, in these patients, IGF-1 was the main independent predictor of both coronary reserve and insulin sensitivity (23). Second, alternatively or additionally, one should consider that the interaction between IGF-1 and its binding proteins is complex. An adequate serum amount of total IGFBP-1 is essential for IGF-1's biological activity (8,25), independently of its phosphorylation status. Moreover, overexpression of IGFBP-1 attenuates IGF-1's growth-promoting actions in vitro but en-

hances them in vivo (26). Thus, a threshold concentration of IGFBP-1 might enhance, rather than impair, IGF-1's bioactivity through a partial agonism-like action.

Because IGFBPs have been characterized only recently and their physiology in humans is not completely understood, we think further studies are needed to better understand the true value of the IGF-1/IGFBP axis in promoting or protecting against vascular complications.

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## IGF Binding Protein-1 and Carotid Intima-Media Thickness in Type 2 Diabetes

Response to Conti et al.

**W**e thank Conti et al. (1) for their interest in our recent article (2) and the editor for the opportunity to further discuss the role of the IGF system in atherosclerosis.

We found an inverse relation between IGF binding protein-1 (IGFBP-1) and carotid intima-media thickness (IMT) in type 2 diabetic patients. In the linear regression model for diabetic subjects with cardiovascular disease (CVD), IGFBP-1 was one of the main determinants of IMT (standardized coefficient  $\beta = -0.353$ ,  $P = 0.027$ ) together with age, Apo B, and pulse pressure; other determinants were diabetes duration, smoking, BMI, sex, and lipoprotein [Lp(a)]. This model explained 57.3% of maximum IMT variation. IGFBP-1 also remained in the linear regression model for IMT in subjects without CVD. Our cross-sectional study setting does not allow the interpretation to which extent these associations are causal. In agreement with previous data (3), IGFBP-1 concentration was inversely related to insulin resistance examined by homeostasis model assessment.

On the contrary, after adjusting for confounders, we found no association between total IGF-I and either IMT or presence of CVD. This indicates the relation between IGFBP-1 and IMT to be independent of IGF-I. Our results underline IGFBP-1 as a marker of insulin resistance and the metabolic syndrome, but this cannot be extrapolated to reflect an IGF-I effect on the vasculature. Similarly, Heald et al. (4) demonstrated an inverse correlation between IGFBP-1, but not IGF-I, and clinical cardiovascular disease in type 2 diabetic subjects.

Another finding was the positive correlation of total IGF-I with LDL cholesterol and Lp(a) and IGFBP-3 with LDL cholesterol, Lp(a), total cholesterol, and Apo B after controlling for age, sex, BMI, and diabetes duration. Thus, in hypercholesterolemic diabetic patients the IGF axis seems to be activated.

The relation of the IGF system to CVD is complex and awaits a conclusive answer after over 1,000 published articles (5). Therefore, we agree with Conti et al. that further research is needed to understand the entire picture.

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## Metformin in Type 1 Diabetes

Is this a good or bad idea?

The article by Meyer et al. (1) revives a debate regarding the appropriateness of metformin use for people with type 1 diabetes. Given the potential for coexisting lactic acidosis and diabetic ketoacidosis, how can one justify its use? Indeed, there was little reason to expect a benefit in patients who were studied: nonobese type 1 diabetic subjects with  $HbA_{1c} < 9.0\%$  who were taking  $\sim 0.7$  units  $\cdot$  kg  $insulin^{-1} \cdot day^{-1}$ . A modest average reduction of daily insulin requirements, 4.3 units, as compared with an increase of 1.7 units for placebo, does not seem to be worth the trade-off of increased risk for severe hypoglycemia (19 events in metformin group vs. 8 events in placebo group). There was no differential effect in terms of  $HbA_{1c}$ . Only 7 of 31

patients (23%) treated with metformin responded in terms of a significant (20%) reduction in insulin requirement. Furthermore, it is likely that the incidence of hypoglycemia would be much greater if more aggressive metabolic targets of  $HbA_{1c}$  had been applied. Despite the failure to observe diabetic ketoacidosis, the limited number and short period of observation does not permit the conclusion that metformin is safe in ketosis-prone diabetic subjects.

We have seen a number of type 1 diabetic patients who have received metformin prescriptions by other practitioners. It appears that these prescriptions were given because of a failure to identify latent autoimmune diabetes in adults or because the physician believed that the potential for insulin dose reduction and lipid improvement justified a putative small risk for diabetic ketoacidosis and lactic acidosis. The temptation to prescribe metformin is increased because of the high prevalence of metabolic syndrome among U.S. adults (2). Indeed, the diagnosis of metabolic syndrome can frequently be made in the type 1 diabetic population. For example, using BMI as a marker for metabolic syndrome, we observed an average BMI of  $27 \text{ kg/m}^2$  in 343 consecutive subjects with type 1 diabetes; this means that our average type 1 diabetic patient is overweight. A BMI  $\geq 30 \text{ kg/m}^2$ , sufficient to diagnosis obesity, was observed in 89 of 343 subjects (26%). Seven of our type 1 diabetic patients had a BMI  $\geq 41 \text{ kg/m}^2$ . Of these severely obese subjects, two were receiving metformin therapy as well as insulin.

The results of the study by Meyer et al. suggest that a small subset of type 1 diabetic patients benefit in terms of insulin dose reduction when metformin is added to insulin. Questions about long-term safety and efficacy in this patient population remain unanswered. Therefore, when is it reasonable and defensible to prescribe metformin in type 1 diabetes? We suggest that metformin should be avoided unless the following criteria are met: 1) insulin resistance is clearly interfering with satisfactory glucose control despite lifestyle interventions; 2) the risk of diabetic ketoacidosis is minimized by an intensive program of insulin, self-monitoring of blood glucose, urine ketone measurement if blood glucose exceeds 300 mg/dl, and regular medical supervision; 3) the patient receives coun-

seling so that he or she understands the potential risk for lactic acidosis; and 4) efficacy is frequently evaluated to justify continued use of metformin.

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## Metformin and Insulin in Type 1 Diabetes

The first step

The insulin-sparing effect of metformin and its positive effect on glucose metabolism is now well documented in insulin-treated type 2 diabetic patients, although the mechanisms of these effects are not overly clear (1). In insulin-treated type 1 diabetic patients, the use of metformin has only been assessed in a few studies and, surprisingly, the same insulin-sparing effect has been found despite some methodological difficulties. However, the clinical interest of metformin in the treatment of type 1 diabetes has remained questionable. For the first time, we have shown in a randomized double-blind trial that metformin has an insulin-sparing effect in type 1 diabetic patients (even if the studied population was not clinically insulin resistant) (2).

The selected patients were C-peptide negative after intravenous glucagon injection and were not identified as having

metabolic syndrome. The patients were moderately overweight (BMI close to 25 kg/m<sup>2</sup>), and their daily insulin needs were not greater than that seen for other type 1 diabetic patients. The insulin-sparing effect of metformin was obtained for basal insulin needs but not for prandial needs. Although no definitive explanation could be given, it is now clear that the use of metformin may be appropriate for some type 1 diabetic patients.

Our study was designed to assess the insulin-sparing effect of metformin in insulin-treated type 1 diabetic patients, and this end was achieved. However, it was not designed to give clinical or biological criteria of indications associated with metformin use and insulin. We found that a subset of patients had a reduction of at least 20% in insulin requirements with stable and satisfactory glucose control, but a backward logistic regression did not allow prediction criteria of a good response to be defined. Thus, an expanded study using selection criteria identified in our previous study coupled with a larger range of BMI or bodyweight and a larger range of daily insulin doses in selected patients must be performed to determine therapeutic indications of this association.

We believe that metformin use could be beneficial in type 1 diabetic patients who are overweight or obese, are receiving large doses of insulin, and have an HbA<sub>1c</sub> >8%. The use of metformin could be very useful in these patients but must be carried out under clinical and biological supervision to avoid complications such as lactic acidosis, which remains exceptional in our experience of type 1 and in type 2 diabetic patients.

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## Prospective Relation of C-Reactive Protein With Type 2 Diabetes

Response to Han et al.

We read with great interest the article by Han et al., which was recently published in *Diabetes Care* (1). The authors studied the association between baseline levels of C-reactive protein (CRP) and the 6-year incidence of the metabolic syndrome and type 2 diabetes in Mexican subjects. They found an odds ratio (OR) of 4.1 (95% CI 2.1–8.0) for developing the metabolic syndrome and an OR of 5.4 (2.2–13.4) for incident type 2 diabetes among women in the highest compared with the lowest tertile of CRP. ORs were adjusted for age, smoking, physical activity, and alcohol intake. In men, no such association was found. Additional adjustment for BMI slightly lowered the ORs; adjustment for waist-to-hip ratio (WHR) did not.

We have studied baseline CRP levels and their relation to incident type 2 diabetes in an age-, sex-, and glucose-stratified sample of the Hoorn Study, a population-based cohort study of glucose tolerance among Caucasian people (2). The study methods and follow-up dura-

tion are closely similar to the Mexico City Diabetes Study (MCDS) described by Han et al., except that our subjects were ~15 years older at baseline. Glucose tolerance status was assessed by a 75-g oral glucose tolerance test at baseline and after 6.4 years of follow-up. CRP was measured in plasma by high-sensitive enzyme-linked immunosorbent assay, while information was available on BMI, WHR, smoking, and physical activity. Of the 140 men and 139 women who had follow-up measurements and were free of diabetes at baseline, 17.8% of the men and 20.9% of the women developed diabetes. In contrast to the findings of Han et al., we did not observe an association between baseline CRP levels and incident diabetes in women, while in men we found an OR of 3.0 (95% CI 1.0–9.3) in the highest compared with the lowest tertile of CRP, after adjustment for age (Table 1). Further adjustment for BMI, smoking, or physical activity did not materially change these results, while adding WHR to the model substantially lowered the OR in men. Thus, in the Hoorn Study, CRP is not a very strong determinant of the development of type 2 diabetes, in contrast to WHR and impaired glucose metabolism (3). We realize that our study sample of 279 subjects is much smaller than the study presented by Han et al. ( $n = 1,244$ ). The relationship in men, however, was strong, whereas in the MCDS it was strong only in women. In the previous prospective study by Barzilay et al. (4) an OR of 2.0 (1.4–2.9) was found when extreme quartiles of CRP were compared. They did not analyze men and women or black and white subjects separately. Pradhan et al. (5) found an OR of 4.2 (1.5–12.0) in women. Both studies did not take WHR into account. Ethnicity

**Table 1—Relative risk associated with high C-reactive protein (second and third tertile compared with the first tertile) for developing type 2 diabetes after 6.4 years of follow-up in the Hoorn Study**

Model	Men		Women	
	2nd tertile	3rd tertile	2nd tertile	3rd tertile
1 Age	1.4 (0.4–4.8)	3.0 (1.0–9.3)	0.7 (0.3–2.1)	1.1 (0.4–3.0)
2 Model 1 + BMI	1.4 (0.4–4.8)	3.0 (1.0–9.3)	0.6 (0.2–1.8)	0.9 (0.3–2.5)
3 Model 1 + WHR	1.4 (0.4–4.8)	1.9 (0.6–6.4)	0.6 (0.2–1.7)	0.9 (0.3–2.5)
4 Model 1 + smoking	1.3 (0.4–4.7)	2.9 (0.9–9.2)	0.7 (0.3–2.1)	1.2 (0.4–3.2)
5 Model 1 + physical activity	1.4 (0.4–4.7)	3.0 (1.0–9.3)	0.7 (0.3–2.1)	1.1 (0.4–3.0)

Data are OR (95% CI).



may also play an important role in explaining the inconsistent results.

In conclusion, we suggest caution in the interpretation of the results because of inconsistent findings, in particular between sexes. It is unclear why inflammation would be important in the pathogenesis of type 2 diabetes only in women or only in men.

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## Prospective Relation of C-Reactive Protein With Type 2 Diabetes

Response to Snijder et al.

We thank Snijder et al. (1) for their interest in our article (2) and for sharing their data relating C-reactive protein (CRP) to the risk of diabetes development in the Hoorn Study. We readily agree that it is unclear why inflammation might be more important to the pathogenesis of type 2 diabetes in women compared with men or indeed vice versa. We have reanalyzed our CRP data using the same covariates as Snijder et al. and essentially our results are unchanged—the relation between CRP and diabetes remains strong in women, even after inclusion of waist-to-hip ratio (WHR), but is absent in men (Table 1). Interestingly, on reanalysis of similarly relevant data from the Insulin Resistance Atherosclerosis Study (IRAS) (3), a study of similar size to the Mexico City Diabetes Study (MCDS), the relation between baseline CRP and diabetes development was also stronger in women compared with men using the same covariate adjustments (data not shown). Moreover, in both the MCDS and IRAS, adjustment for BMI at-

tenuated the link between CRP and diabetes more so than WHR.

One possibility of the divergent results could be the younger baseline age (~46 years) in MCDS in comparison to that of the Hoorn study (~61 years). In this respect it is important to consider the additional potential confounding influence of hormonal replacement therapy (HRT) in women. HRT use increases CRP concentrations in women (4), but paradoxically recent data from the Heart and Estrogen/Progestin Replacement Study suggest that HRT reduces risk of diabetes development by 35% (5). In MCDS, only 4% of women were on HRT at baseline and hormonal use did not confound the link between CRP and diabetes. However, because of higher age, a potentially much higher proportion of women in the Hoorn Study may have been on HRT at baseline, which could thus disguise any potential association between CRP and diabetes risk.

We acknowledge that our inability to find an association between CRP and diabetes risk in men may simply be due to a lack of power. A recent relevant study (6) from the West of Scotland Coronary Prevention Study (WOSCOPS) group that included 127 new cases (predominantly Caucasian) of diabetes reported a strong association between baseline CRP and diabetes risk independent of BMI, blood pressure, smoking, and fasting lipids and glucose concentrations. Alternatively and as Snijder et al. suggest, ethnicity may also play a role in explaining inconsistent results.

Clearly, further studies are needed to examine the relation between inflamma-

**Table 1—ORs (with 95% CIs) associated with high C-reactive protein (second and third tertile compared to first tertile) for developing type 2 diabetes after 6.5 years of follow-up in the Mexico City Diabetes Study**

Model	Men (35 incident cases/n = 460)		Women (54 incident cases/n = 672)	
	Second tertile (0.91–1.64 ng/ml)	Third tertile (>1.64 ng/ml)	Second tertile (1.24–2.16 ng/ml)	Third tertile (>2.16 ng/ml)
1 Age	0.7 (0.3–1.7)	1.0 (0.5–2.3)	3.1 (1.2–7.9)*	5.7 (2.3–13.9)†
2 Model 1 + BMI	0.6 (0.2–1.4)	0.8 (0.4–1.9)	2.2 (0.9–5.8)*	3.1 (1.2–8.1)*
3 Model 1 + WHR	0.6 (0.2–1.5)	0.9 (0.4–2.1)	3.0 (1.2–7.7)*	5.4 (2.2–13.3)†
4 Model 1 + smoking	0.7 (0.3–1.7)	1.0 (0.5–2.3)	3.1 (1.2–8.0)*	5.7 (2.3–14.1)†
5 Model 1 + physical activity	0.7 (0.3–1.6)	0.9 (0.4–2.0)	2.7 (1.0–7.1)*	5.3 (2.2–13.2)†

The main effect of gender is significant ( $P < 0.05$ ) for each model. Gender-CRP tertile interaction: \* $P < 0.05$ , † $P < 0.01$ .

tory markers and diabetes risk. It would be useful if such studies were to report associations separately in men and women in order to enable identification of any potential gender differences.

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## A Multivariate Logistic Regression Equation to Screen for Diabetes

Response to Tabaei and Herman

We read the article by Tabaei and Herman (1) in the November 2002 issue of *Diabetes Care* with great interest and feel that it covers an exciting and vital area of diabetes research. We applaud the authors' attempts to improve current standards of diabetes screening and the journal's willingness to publish important new findings in the field. However, when we reviewed the article, we found the results difficult to reproduce, especially as there were no numerical examples provided in the text. Using the precise formula given in the article, our calculations produced negative probability values. We would therefore like to take this opportunity to point out a source of possible error in the equation and clarify the logistic regression model.

The logistic regression model and the "logit" model can be written as:

$$\ln[P/(1 - P)] = \beta_0 + \beta_i X_i + e \quad (2)$$

where  $X = \beta_0 + \beta_i X_i$  are the coefficients (parameters) for the linear predictor and  $X_i$  represents the values of the explanatory variables (we have assumed that these coefficients have been correctly estimated in the article);  $\ln$  is the natural logarithm,  $\log_{\exp}$ , where  $\exp = 2.71828$ . . . ;  $P$  is the probability that the event "previously undiagnosed diabetes" occurs;  $P/(1 - P)$  is the "odds ratio"; and  $\ln[P/(1 - P)]$  is the log odds ratio, or "logit."

In contrast to the simple linear probability model ( $Y = a + bX + e$ ), the logit distribution constrains the estimated probabilities to lie between 0 and 1. For instance, the estimated probability is:

$$P = [\exp(\beta_0 + \beta_i X_i)] / [1 + \exp(\beta_0 + \beta_i X_i)]$$

or

$$P = 1/[1 + \exp(-\beta_0 - \beta_i X_i)]$$

For a 45-year-old man with a BMI of 29 kg/m<sup>2</sup>, a plasma glucose level of 125 mg/dl, and a postprandial time of 3 h, the probability calculated with the published formula would be  $P = -0.0555$  (i.e.  $P < 0$ ). This result does not match the requirement for probabilities to lie between 0 and 1. For the same case, but with the corrected formula, the probability of being previously undiagnosed for diabetes is  $P = 0.04996$ .

## Details of the calculation with the formula published in the article:

$$P = 1/[1 - \exp(-X)]$$

$X = -10.0382 + 0.0331 \times (\text{age} = 45 \text{ years}) + 0.0308 \times (\text{plasma glucose} = 125 \text{ mg/dl}) + 0.25 \times (\text{postprandial time} = 3 \text{ h}) + 0.562 \times (\text{sex} = 0) + 0.0346 \times (\text{BMI} = 29 \text{ kg/m}^2)$ .  $X = -2.9453$  and  $P = 1/[1 - \exp(-X)] = 1/(1 - \exp(2.9453)) = 1/(1 - 19.01636644) = 1/-18.01636644$ .  $P = -0.0555$ .

## Details of the calculation with the corrected formula:

$$P = 1/[1 + \exp(-X)]$$

$X = -2.9453$ ;  $P = 1/[1 + \exp(-X)] = 1/(1 + \exp[2.9453]) = 1/(1 + 19.01636644) = 1/20.01636644$ ;  $P = 0.04996$ .

Therefore, we would suggest that the following equations should be used:

$X = -10.0382 + 0.0331 \times (\text{age}) + 0.0308 \times (\text{plasma glucose}) + 0.25 \times (\text{postprandial time}) + 0.562 \text{ (if female)} + 0.0346 \times (\text{BMI})$   
and  $P = 1/[1 + \exp(-X)]$

We hope these comments serve to clarify this logistic regression equation to screen for diabetes and, in conjunction with the valuable work of our colleagues Drs. Tabaei and Herman, help expand the knowledge base in this key area of diabetes research.

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## A Multivariate Logistic Regression Equation to Screen for Diabetes

Response to Roze, Palmer, and Valentine

We would like to thank Roze, Palmer, and Valentine (1) for their careful reading of our article (2) entitled “A multivariate logistic regression to screen for diabetes: development and validation.” The estimated probability formula for the logistic regression model was indeed misprinted with a minus sign. The correct estimated probability formula is:

$P = 1/(1 + e^{-x})$ , where  $x = -10.0382 + [0.0331 (\text{age in years}) + 0.0308 (\text{random plasma glucose in mg/dl}) + 0.2500 (\text{postprandial time assessed as 0 to 8+ hours}) + 0.5620 (\text{if female}) + 0.0346 (\text{BMI})]$ .

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## β2-Adrenergic Receptor Polymorphism and Obesity in Type 2 Diabetes

Response to van Tilburg et al.

We read with interest the observation letter by van Tilburg et al. (1) reporting that the Glu27Gln polymorphism of the β2-adrenergic receptor (B2ADR) has no important effect on BMI, metabolic control, or plasma lipids. However, the authors did not mention the physical activity and diet regimen in their study. B2ADR is a major lipolytic receptor in human adipocytes, activated by catecholamines especially during a weight reduction regimen. Meirhaeghe et al. (2) reported that B2ADR Glu27Gln polymorphism is significantly associated with obesity in individuals who do not have regular physical activity, while no effect of such polymorphism is found in those who do have physical activity, and suggested that obese individuals with B2ADR Glu27Gln genotype may benefit from physical activity to reduce their body weight. We also demonstrated the same association between another B2ADR polymorphism (Arg16Gly) and obesity in individuals treated with a combined low-energy diet and exercise regimen (3). Therefore, the authors should have further divided the subjects according to the degree of their physical activity and analyzed the data, because body weight and body fat or metabolic control are influenced by physical activity and dietary changes, especially in individuals with such polymorphisms.

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## β2-Adrenergic Receptor Polymorphism and Obesity in Type 2 Diabetes

Response to Yoshioka, Yoshida, and Yoshikawa

We recently reported that our group ( $n = 502$ ) of type 2 diabetic subjects did not reveal statistically significant differences in BMI with respect to the three groups of carriers of β2-adrenergic receptor polymorphisms (1). Meirhaeghe et al. (1) report that in men not participating in physical activity, Glu27Gln carriers had a higher BMI than the Glu27 (combined heterozygous and homozygous) carriers ( $27.2 \pm 0.4$  vs.  $25.2 \pm 0.3$  kg/m<sup>2</sup>), while no effect on BMI was found in the men not participating in physical activity.

We have not collected data on the physical activity of our subjects. However, taking into account that our subjects had a mean age of  $61 \pm 9$  years (roughly 10 years older than the subjects reported by Meirhaeghe et al.), we suspect that the vast majority of them do not participate in much physical activity. In any case, their physical activity is presumably less than that of the subjects studied by Meirhaeghe et al. Much to our surprise, we actually



find the opposite, which is a lower (and not a higher!) BMI ( $27.2 \pm 3.8$ ) in the Gln27Gln carriers compared with the others ( $27.9 \pm 4.4$  for Gln27Glu and  $28.2 \pm 4.0$  kg/m<sup>2</sup> for Glu27Glu), although the differences did not attain statistical significance. These findings were similar for men and women when taken together or separately.

Obviously, there are two major differences between these two studies. The subjects in our study (2) had type 2 diabetes and were considerably older. Whether these two differences explain the divergent findings of the study of Meirhaghe et al. and our study is uncertain. However, the suggestion by van Tilburg et al. that, if anything, the BMI was lower (and not higher) in the Gln27Gln27 group might

point to a chance finding in the studies by Meirhaghe et al. As for the observations regarding the Arg16Gly polymorphism, we have not studied this polymorphisms in our group of subjects.

It has become more and more clear that association studies of a particular polymorphism with a certain physiological parameter are often hard to replicate; this necessitates the need to perform replication studies before a finding can be regarded as truly positive.

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